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(71) Applicant *(for all designated States except US)*: ORTHO PHARMACEUTICAL CORPORATION [US/US]; Route 202, Raritan, NJ 08869-0602 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : KLOETZER, William, S. [US/US]; 803 Summerhill Court, Encinitas, CA 92024 (US). NASO, Robert, B. [US/US]; 2640 Vistosa Place, Carlsbad, CA 92008 (US). WARNER, John, R. [US/US]; 4125 Pilon Point, San Diego, CA 92130 (US).

(74) Agent: MINIER, Robert, L.; One Johnson & Johnson Plaza, New Brunswick, NJ 08933-7003 (US).

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(54) Title: IMMUNOSUPPRESSIVE PEPTIDES AND METHODS OF USE

(57) Abstract

Novel peptide sequences useful for vaccinating cats against feline leukemia virus and humans against human retroviruses, for detecting the presence of a protein having an apparent molecular weight of about 35,000 daltons and a protein having an apparent molecular weight of about 110,000 daltons, both of which are expressed by mammalian leukemia and cancer cells, and related therapeutic applications.

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IMMUNOSUPPRESSIVE PEPTIDES AND METHODS OF USE

Background of the Invention

Immunosupression, nonregenerative anemia and neutropenia are prominent clinical symptoms of animals persistently infected with retroviruses. Feline leukemia virus (FLV) is a leukemia-inducing retrovirus which can also induce aplastic anemia and immunosupression in persistently viremic cats. Human acquired immunodeficiency syndrome (AIDS) is caused by retroviruses called Human Immunodeficiency Viruses, e.g., HIV-I and HIV-II, referred to herein collectively as HIV. HIV also causes immune suppression in viremic individuals. The retrovirus-mediated immunosuppression in feline leukemia and human AIDS may, in fact, result in susceptibility to opportunistic infections. It is these infections that, in the immunosuppressed individual, most often are the cause of morbidity and mortality. In FLV-induced disease, the immunosuppression appears to be attributed, at least in part, to the viral gene product called p15E, i.e., the viral transmembrane protein. In AIDS, immunosuppression may be a result of a complex series of events related to HIV replication in helper T-cells. The presence of immunosuppressive peptides in HIV (as described below), however, suggest that gp41, the HIV immunosuppressive analog of p15E, may also play a role in immunosuppression associated with the initial establishment of a HIV infection.

A 17 amino acid synthetic peptide from the murine leukemia virus (MLV) p15E sequence has been shown by others to exert *in vitro* immunosuppressive effects similar to those induced by the complete viral protein or the intact virus (Cianciolo, et al., Science 230:453, 1985). One measure of

this immunosuppressive effect is the in vitro inhibition of mitogen-induced lymphocyte proliferation. The peptide disclosed by Cianciolo et al. was derived from MLV and has the amino acid sequence LQNRRGLDLLFLKEGGL. Comparison of this sequence with sequences of the transmembrane protein of several other retroviruses showed that this region is relatively well conserved among retroviruses including FLV, Human T-cell Lymphotropic virus (HTLV-I and II), bovine leukemia virus (BLV), and to a much lesser degree HIV. Cianciolo, et al. also reported the murine p15E peptide to be an active immunosuppressant only if it was carbodiimide crosslinked to bovine serum albumin (BSA). Reportedly, unconjugated peptide showed no activity.

Monoclonal antibodies to an uncharacterized antigenic determinant of p15E which detect, by fluorescence-activated cell sorting, antigen in various cell lines derived from human cancers have also been described (J. Exp. Med. 159:964, 1984). However, the biochemical nature of the p15E-related antigen was not disclosed and no relationship has been shown between it and either the 35,000 dalton protein or the 110,000 dalton protein expressed by various human leukemia and cancer cell lines and described herein. Although the monoclonal antibody can be used to absorb and remove in vitro suppressive activity, there have been no reported results indicating that these antibodies bind to the highly conserved immunosuppressive region of viral p15E or that such binding blocks immunosuppression. Indeed, the authors have orally stated at a recent conference that the antibodies do not bind to the 17 amino acid peptide from the conserved region of p15E. Recent reports have also shown that a biologically active p15E-related antigen is released by cell lines derived from human tumors (J. Immunol. 137:2726, 1986).

Human leukemia is often characterized by hematopoietic suppression that in some ways is similar to the immunosuppression mediated by retroviruses. Cell lines derived from human leukemias, i.e., K562 cells and HL-60 cells, have been reported to secrete factors which block in vitro differentiation of normal bone marrow cells and mitogen induced proliferation of spleen cells. It has also been reported that treatment of certain of these cell lines, i.e., K562 cells and HL-60 cells, in vitro with cytodifferentiating agents diminishes the expression of the suppressive factors. For additional information, incorporating reference is made to the following:

Olofsson, T., Olsson, I. (1980) Suppression of normal granulopoiesis in vitro by a leukemia associated inhibitor (LIA) derived from a human promyelocytic cell line (HL-60). Leukemia Res 4:437;

Olofsson, T., Nilsson, E., Olsson, I. (1984) Characterization of the cells in myeloid leukemia that produce leukemia associated inhibiter (LAI) and demonstration of LAI-producing cells in normal bone marrow. Leukemia Res. 8:387;

Steinberg, H.N., Tsiftsoglou, A.S., Robinson, S.H. (1985), Loss of suppression of normal bone marrow colony formation by leukemic cell lines after differentiation induced by chemical agents. Blood 65:100; and

Chiao, G.W., Heil, M., Arlin, Z., Lutton, J.D., Choi, Y.S., Leung, K. (1986), Suppression of lymphocyte activation and functions by a leukemia cell-derived inhibitor, PNAS 83:3432.

It is an object of the present invention to provide

antibodies which react with the immunosuppressive peptide region of p15E and with p15E itself.

It is yet another object to develop a peptide capable of being used as a vaccination against FLV.

It is still yet another object to provide immunosuppressive peptides and antibodies thereto for therapeutic applications.

Still another object of the present invention is to provide an antibody for detecting p15E-related immunosupressive factors whether virally, non-virally, or endogenous virus induced.

Summary of the Invention

Novel immunosuppressive peptide sequences which, when conjugated to a carrier molecule, are capable of being used as therapeutic agents in the treatment of immune-related dysfunctions such as autoimmune diseases, graft rejection, and allergies are provided. Also provided are the use of these peptides as vaccines to prevent immunosuppression and disease upon exposure to retroviruses such as FLV, BLV, HTLV, and HIV. Further provided are antibodies to the suppressive peptide(s) which are capable of diagnosing human cancer by identifying in an immunoassay the expression of p15E-related proteins.

The present invention also provides a soluble, immunosuppressive peptide which is biologically active without being cross-linked to a carrier molecule. One peptide included within p15E (designated I6B) and having the amino acid sequence AKLRERLKQRQQ displays an unusual adhesive property. Antiserum to the I6B peptide was found to neutralize virus infectivity. Upon blocking antiserum with the I6B peptide (which should abrogate antibody-mediated neutralization) enhanced viral infectivity was detected. More recently, immunocytochemical studies with I6B antiserum have detected antigen in FLV-infected cells. Blocking of this antiserum with the I6B peptide (which should prevent antibody recognition of antigen) enhanced the cellular staining in an apparent non-specific manner.

Another novel peptide included within p15E (designated ISP) and having the amino acid sequence LQNRRGLDILFLQEGGLC also displays immunosuppressive activity.

The present invention provides an immunosuppressive

polypeptide, designated δI6B:ISP, which comprises a portion of the I6B domain synthetically linked to the ISP domain and having the following amino acid sequence:

A K L R E L K Q R Q Q L Q N R R G L D I L F L Q E G G L C
|------δI6B-------| |--------------ISP----------------|

δI6B:ISP is a soluble polypeptide which inhibits in vitro mitogen-induced proliferation of murine splenic T lymphocytes. The observed inhibition proliferation appears not to be a function of cell toxicity. Other peptide configurations which are also active include related sequences from retroviral and cellular proteins, inverted structures (ISP:I6B), truncated I6B:ISP and ISP:I6B peptides in monomeric, dimeric and multimeric forms. The advantages of these peptides over carrier molecule-linked peptides include higher solubility, lower immunogenicity and biological activity at lower (w/v) concentrations.

The present invention also concerns a purified polypeptide having a molecular weight of about 110,000 daltons which is detected on mammalian leukemia and cancer cells. This polypeptide is detected with antiserum raised to an immunosuppressive polypeptide from the predicted amino acid sequence of feline leukemia virus p15E. Antibodies which detect this 110,000 dalton polypeptide are useful for diagnosing numerous malignancies and for monitoring peripheral blood of remission leukemia patients for residual disease. Moreover, because this polypeptide plays a functional role in leukemia and tumor cell-mediated subversion of cancer immunity, antibodies directed to it or its receptor, as well as synthetic peptides derived from its predicted amino acid sequence, have therapeutic applications for modulating suppressed tumor immunity.

Polyclonal rabbit antisera raised to biologically-active KLH conjugates of feline, human T cell and murine leukemia viral suppressive peptides (ISP) have been generated and affinity purified to obtain FLV-ISP antibodies. Immnocytochemical staining with such FLV-ISP antibodies strongly detects antigen in FLV-infected cat cells. However, the ISP antibodies also detect an antigen in diverse cell lines established from human leukemia patients but not in normal murine fibroblasts. Antibody staining of peripheral blood leukocytes (PBL) from normal individuals is very weak compared to leukemia cells or phytohemagglutinin (T lymphocyte mitogen) stimulated PBLs. Immunoprecipitation from FL74 cells metabolically labeled for 4 hours with [^{35}S]methionine detected viral p15E, viral gPr85^{env} and the novel polypeptide of the present invention having a molecular weight of about 110,000 daltons. A similar experiment with the human leukemia cell line Raji detected three proteins having molecular weights of about 35,000 (35K), 37,000 (37K) and 110,000 (110K) daltons. The 110K protein, but not the 35K or the 37K protein, was detected in human leukemia cell lines K562, EM2 and HL60. The 110K protein was also found in the human epidermoid carcinoma cell line A431 but not in normal murine 3T3 fibroblasts. Using centrifugation speeds that sediment virus-associated p15E, the 110K protein from human leukemia cells was detected in the supernatant fraction of conditioned growth medium. This soluble 110K protein, which is released into growth media by cell lines established from diverse leukemias and solid tumors, displays an epitope similar in structure and function to the immunosuppressive peptide domain of FLV p15E.

Brief Description of the Figures

Figure 1. δI6B:ISP Suppression of Murine T Cell Proliferation

Balb/c spleen cells were cultured for 4 days in the presence of the murine T cell mitogen Con A (4 μg/ml) and titrated amounts of δI6B:ISP peptide. Proliferation of mitogen-stimulated cultures exposed to the δI6B:ISP peptide is presented as a percentage of control cultures (cells and mitogen only). The dashed line indicates proliferation of positive control cultures (cells + mitogen only).

Figure 2. Comparison of Suppressive Activity of the I6B, ISP, and δI6B:ISP Peptides

Balb/c spleen cells were cultured for 4 days in the presence of Con A and titrated amounts of these three peptides. The dashed line indicates stimulation of control cultures (cells + mitogen only).

■—■ = I6B
●—● = ISP
▲—▲ = δI6B:ISP

Figure 3. δI6B:ISP Induced Immunosuppression

Balb/c spleen cells were stimulated with either Con A (A) or LPS (B) in the presence of titrated amounts of the δI6B:ISP peptide. Cell cultures were incubated for either 4 or 5 days before harvesting. (A) Effect on T Cell Proliferation. (B) Effect on B Cell Proliferation.

▲-▲ = Day 4
●-● = Day 5

Figure 4: Suppression of lymphoid cell proliferation

Balb/c spleen cells were cultured in the presence of Con A and titrated amounts of δI6B:ISP peptide for either 3, 4, or 5 days before harvesting. The proliferation values are a percentage of control cultures (cells with Con A only).

●—● = Day 3
■—■ = Day 4
▲—▲ = Day 5

Figure 5: Antibody Binding to the δI6B:ISP Peptide

The δI6B:ISP peptide was bound to microtiter wells and incubated with different anti-peptide antibody preparations in a standard enzyme-linked immunosorbent assay (ELISA). AP558-28, affinity purified rabbit antiserum against the ISP peptide; 9-14F2-3, mouse monoclonal antibody against the ISP peptide; R112-5, rabbit antiserum against the I6B peptide: AP67, affinity purified rabbit antiserum against the c-abl oncogene product (negative control).

●—● = α - ISP (AP55-28)
▲—▲ = α - ISP (9-14F2-3)
▼—▼ = α - I6B (R112-5)
○—○ = α - abl (AP67)

Detailed Description of the Invention and Specific Embodiments

The following table lists amino acids and abbreviations used within the present application:

Amino Acid	3-Letter	1-Letter
L-tyrosine	tyr	Y
glycine	gly	G
L-phenylalanine	phe	F
L-methionine	met	M
L-alanine	ala	A
L-serine	ser	S
L-isoleucine	iso	I
L-leucine	leu	L
L-threonine	thr	T
L-valine	val	V
L-proline	pro	P
L-lysine	lys	K
L-histidine	his	H
L-glutamine	gln	Q
L-glutamic acid	glu	E
L-tryptophan	trp	W
L-arginine	arg	R
L-aspartic acid	asp	D
L-asparagine	asn	N
L-cysteine	cys	C
unspecified	-	X

The present invention provides an immunosuppressive peptide which comprises a peptide having less than about 40 amino acid residues, said amino acid residues comprising an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being selected from amino acid sequences included within the amino acid sequence

LQNRRGLDILFLQEGGLCAALKEECCF. Within this application, "peptide" means a chain of amino acid residues having between 2 and about 100 amino acid residues, and includes peptides which are purified from naturally occuring products, or produced by synthetic or recombinant DNA methods. Amino acid chains having greater than about 100 amino acid residues are referred to herein as polypeptides. In one embodiment of the invention, the peptide is conjugated to a carrier molecule so as to form an immunosuppressive compound. Within this application, "carrier molecule" means a molecule which, when conjugated, e.g., cross-linked, to a ligand of interest, either increases the immunogenicity of the ligand or activates the biological activity, e.g., immunosupressive activity, of the ligand. Carrier molecules which increase the immunogencity of ligands are known in the art and include large proteins such as keyhole limpet hemocyanin (KLH), ovalbumin, porcine thyroglobulin, and bovine serum albumin (BSA), lipids, and peptides. Carrier molecules which activate the biological activity of ligands are also known in the art, e.g., polyethylene glycol. Methods for conjugating peptides to carrier molecules are known in the art and are described in Lerner, et al., PNAS (USA) (1981) 78: 3403-3407, Church, et al., PNAS (USA) (1983) 80:250, and Erlanger, Meth of Enzmology (1980) 70:85-104. Coupling agents useful for preparing peptide: carrier molecule conjugates are known in the art and include conventional cross-linking agents such as aldehydes, e.g., glutaraldehyde, carbodiimides, succinimides, bis-diazotized benzadines, and imidates. Applicants also contemplate the addition of certain amino acids, i.e., cysteine or lysine, to either the carboxy terminus or the amino terminus of the peptides of the present invention for the purpose of cross-linking to a carrier molecule. In a preferred embodiment of the invention the carrier molecule is keyhole

limpet hemocyanin. In another preferred embodiment of the invention the carrier molecule is conjugated to the peptide with glutaraldhyde.

The present invention also provides an immunosuppressive compound which comprises a peptide having less than about 40 amino acid residues, said amino acid residues comprising an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being selected from amino acid sequences included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
QREKRAVGIGALFLGFLG,
QLTVWGIKQLQARIL
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
AKLRERLKQRQQ,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ, and
LGSRRTLMLLAQMRKIS,

said peptide being conjugated to a carrier molecule.

The present invention provides still further

immunosuppressive peptides which comprise a peptide having less than about 40 amino acid residues, said amino acid residues comprising an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being selected from amino acid sequences included within the amino acid sequence QREKRAVGIGALFLGFLG or the amino acid sequence QLTVWGIKQLQARIL. Yet another immunosuppressive peptide is provided which comprises an antigenic determinant homologous to an antigenic determinant of the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC. Within this application, an antigenic determinant is homologous to another antigenic determinant if each antigenic determinant binds to the same antibody.

Further yet, the present invention provides an immunosuppressive peptide which comprises a first amino acid sequence of at least 5 amino acid residues, said first amino acid sequence being selected from amino acid sequences included within the amino acid sequence AKLRERLKQRQQ, and at least one other amino acid sequence of at least 5 amino acid residues, said other amino acid sequence being selected from amino acid sequences included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,

LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

The peptides provided by the present invention may be cyclic or may comprise repeating units of a polymer or a dimer.

In one embodiment of the invention, the amino terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ is linked to the carboxy terminus of the amino acid sequence included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,

LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

In another embodiment of the invention, the carboxy terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ is linked to the amino terminus of the amino acid sequence included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

In still another embodiment of the invention, the carboxy terminus and the amino terminus of the amino acid sequence

included within the amino acid sequence AKLRERLKQRQQ are each linked to an amino acid sequence included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

The amino acid sequences linked to the carboxy terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ may be the same or may be different from the amino acid sequence linked to the amino terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ. In a preferred embodiment of the invention, the immunosuppressive peptide comprises the amino acid sequence AKLRERLKQRQQLQNRRGLDILFLQEGGLC. In another preferred embodiment of the invention the immunosuppressive peptide comprises the amino acid sequence

AKLRELKQRQQLQNRRGLDILFLQEGGLC.

The present invention also provides a purified polypeptide which comprises an antigenic determinant for which an antibody generated against the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC has affinity, the polypeptide being expressed in mammalian cancer cells. Within this application, an antibody having affinity for an antigenic determinant means the antibody is capable of binding to the antigenic determinant. In one embodiment of the invention, the purified polypeptide has an apparent molecular weight of about 110,000 daltons. In another embodiment of the invention, the purified polypeptide has an apparent molecular weight of about 35,000 daltons.

Also provided are purified nucleic acid sequences which encode each of the purified polypeptides provided herein. Such nucleic acid sequences, including DNA, RNA, and cDNA sequences, are useful for preparing expression vectors capable of expressing the purified polypeptides of the present invention. Additionally, antibodies which have affinity for each of the purified the polypeptides of the present invention are provided. In one embodiment of the invention the antibody is a polyclonal antibody. In another embodiment of the invention the antibody is a monoclonal antibody.

Further still, the present invention provides a purified protein which affinity for the purified polypeptides of the present invention. This purified protein is present on the surface of hematopoietic cells.

A therapeutic composition which comprises an antibody of the present invention and a pharmaceutically acceptable carrier is also provided. This therapeutic composition is

useful in a method for treating an immunologically or hematopoietically suppressed subject by administering to the subject an effective, immunosuppressing blocking amount of the therapeutic composition.

Yet another therapeutic composition is provided by the present invention. This therapeutic composition comprises a purified polypeptide of the present invention, or a peptide having an amino acid sequence of at least 5 amino acids, said amino acid sequence being included within the purified polypeptide, and a pharmaceutically acceptable carrier. This therapeutic composition is useful in a method for suppressing the immune system of a subject by administering to the subject an effective immunosuppressing amount of the therapeutic composition.

Still further, a therapeutic composition is provided which comprises a portion of the purified protein of the present invention having immunosuppressive peptide-binding activity and a pharmaceutically acceptable carrier. This therapeutic composition is useful in a method for treating an immunologically or hematopoietically suppressed subject by administering to the subject an effective, immunosuppressing blocking amount of the therapeutic composition.

A method for detecting a cancer cell in sample, e.g., a whole blood sample or bone marrow sample, is provided which comprises detecting a cell from the sample which expresses a polypeptide having an antigenic determinant which binds to an antibody raised to the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC, said polypeptide being expressed in mammalian cancer cells.

Additionally, a method for diagnosing cancer in a subject

is provided which comprises detecting a polypeptide having an antigenic determinant which binds to an antibody raised to the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC, said polypeptide being expressed in mammalian cancer cells, or a portion of said polypeptide which includes said antigenic determinant, in a body fluid sample taken from the subject.

Further yet is provided a vaccine which comprises an immunosuppressive peptide of the present invention and a pharmaceutically acceptable carrier. This vaccine is useful for immunizing a subject against a retroviral infection by administering to the subject an effective immunizing amount of the vaccine.

Still yet another vaccine is provided which comprises an immunosuppressive compound of the present invention and a pharmaceutically acceptable carrier. This vaccine is useful for immunizing a subject against a retroviral infection by administering to the subject an effective immunizing amount of the vaccine.

The present invention further provides a therapeutic composition which comprises an immunosuppressive peptide of the present invention and a pharmaceutically acceptable carrier. This therapeutic composition is useful for suppressing the immune system of a subject by administering to the subject an effective immunosuppressing amount of the therapeutic composition.

Finally, a therapeutic composition is provided which comprises an immunosuppressive compound of the present invention and a pharmaceutically acceptable carrier. This therapeutic composition is useful for suppressing the immune system of a subject by administering to the subject

an effective immunosuppressing amount of the therapeutic composition.

Applicants have shown that ISP, synthetically produced and cross-linked with glutaraldehyde to keyhole limpet hemocyanin (KLH), suppresses mitogen-stimulated T-cells in vitro, while KLH alone shows no immunosuppressive activity. As with the peptide reported by Cianciolo, ISP was not immunosuppressive when unconjugated to a carrier molecule.

Synthetic ISP, crosslinked to KLH with gluteraldehyde was also formulated with Freund's adjuvant and used to generate a rabbit polyclonal antiserum using standard immunization techniques. It was observed that this antiserum recognized ISP specifically in a standard ELISA immunoassay using ISP as immobilized antigen and peroxidase-labeled anti-rabbit antibody.

Unexpectedly, it was also observed that the antiserum reacted strongly with FLV p15E and its precursor polyprotein (gPr85env) from lysates of FLV-infected cells analyzed by standard Western blot. This was surprising since antibody to peptides do not often recognize native proteins, or if they do, they generally do so only weakly. Immunocytochemical analysis with peptide affinity-purified antibody confirmed the specificity of the antibody for antigen in FLV-infected cells since incubation of antibody with ISP in solution was found to block reactivity of the antibody with the p15E-related proteins.

Peptide affinity purified rabbit antibody to ISP was used to screen by Western blot analysis protein antigens derived from cell lysates of several human leukemia cell lines not overtly or exogenously infected by retroviruses. Included

in the study were K562, EM2, CEM and Raji human leukemia cells. Surprisingly, the antibody also reacted with and specifically identified a protein or proteins with an apparent molecular weight of about 35,000 daltons in each of these cells. This protein was designated p35. Normal human peripheral blood leukocytes and normal fibroblasts do not contain this protein(s) and thus did not provide binding sites for the anti-peptide antibodies.

Thus, the antibodies to ISP of the present invention are useful for detecting p15E-related proteins in human leukemia cells and for diagnosing a variety of naturally occurring human cancers in which p15E-like proteins are expressed. Furthermore, since cells infected with retroviruses also express p15E-related proteins, e.g., FLV-infected cells, antibody to ISP or related sequences is useful for detecting viral antigen in tissues or body fluids containing such viruses and for diagnosing retrovirus-induced diseases such as HIV-induced AIDS, HTLV-induced leukemia, FLV-induced feline leukemia, BLV-induced bovine leukemia, and other as yet unknown diseases induced by endogenous retroviral sequences.

Based upon the antigenic homology between retroviral p15E and the p35 protein(s) that react with antiserum to ISP, applicants maintain that p35, like p15E, is an immunosuppressive protein or set of related proteins. This link between immunosuppression and p35 may be deduced from studies on a human tumor cell line designated K562. K562 cells were derived from a chronic myelogenous leukemia (CML) patient in erythroleukemic blast crisis. The established cell line is characterized by the expression of a cancer gene called bcr-abl. The expression of this gene is a result of an exchange of genetic information between chromosomes in the patient resulting in the formation of an

aberrant chromosome called the Philadelphia chromosome. Formation of this altered chromosome results in the expression of a hybrid gene composed of so-called bcr sequences and abl sequences. Expression of this gene is thought to result in chronic myelogenous leukemia as the abl gene is a known oncogene.

Antisera directed to components of bcr and abl proteins have been developed and have been used to identify the bcr-abl hybrid gene product (called $P210^{bcr-abl}$ or P210) in these K562 cells (Nature 315:550, 1985). P210 is known to possess kinase enzymatic activity. P210 can be detected by its ability to autophosphorylate while in a complex with antibody (an immune complex). Treatment of K562 cells with certain drugs such as phorbol 12-myristate 13 acetate (PMA) and mezerein induces a change in expression of the activated oncoprotein P210 such that intracellular expression of P210 is drastically reduced. Treatment with these agents also causes differentiation of K562 cells and a loss of transformed phenotype.

As described above, K562 is one of the human cell lines that contains p35 as shown by detection with the anti-ISP antibody of the present invention. In addition to its effect on P210 expression, treatment of K562 cells with PMA also causes a drastic reduction in p35 levels. Thus it appears that p35 expression is directly correlated with the expression of the CML oncogene P210. As stated above, K562 cells and other established cell lines derived from human myeloid leukemia patients have been shown to secrete several factors which inhibit <u>in vitro</u> growth of normal bone marrow cells (Olofsson and Olsson, Leukemia REs 4:437, 1980; and 8:387, 1984; Steinberg et al., Blood 65:100, 1985) and mitogen induction proliferation of peripheral blood lymphocytes (Chiao, et al., PNAS 83:3432, 1986). It

has also been shown that expression of these suppressive factors diminishes in K562 cells upon induction of differentiation with hemin. These results indicate that p35 is the suppressive factor previously described to be secreted by K562 cells and that p35 is involved in the disease state of K562 cells. A direct correlation of p35 expression and P210 enzyme activity has also been observed in CML EM2 cells (W. Kloetzer, unpublished results). In this cell line both P210 and p35 levels are enhanced upon treatment with PMA and the cells do not loose their transformed phenotype. EM2 cells are derived from a patient in myeloid blast crisis. These findings suggest that CML cells of different cell lineage respond to PMA differently, but the results also confirm the relationship between oncogene expression and p35 expression.

Based upon the correlation between p35 expression and expression of an activated cellular oncogene, p35 represents a target for development of new therapeutics or vaccines. Antibodies to p35 or antibodies to immunosuppressive peptides derived from p15E or p35 sequences will therefore have value in blocking the suppressive effects of these p15E-related proteins in human tumors and in retroviral induced diseases. Alternatively, synthetic peptide analogues will block the suppressive effects of the suppressive proteins. Such analogues compete for immunosuppressive peptide receptors, thereby blocking binding of the immunosuppressive protein, but are biologically inactive because they cannot by themselves initiate the sequence of events to immunosuppression.

To exemplify the value of immunosuppressive p15E-related peptides as vaccines for prevention of retroviral induced disease, applicants immunized four cats with ISP conjugated to KLH with gluteraldehyde and formulated in Freund's

Adjuvant. Three of the four cats were protected from viremia upon challenge with FLV while six of eight control cats became viremic. These results indicate that ISP is useful as an effective vaccine in the prevention of FLV viremia.

Applicants have further identified three peptides derived from HIV sequences that are capable of suppressing mitogen induced proliferation of T-cells in vitro. One peptide (designated SUP2) with the amino acid sequence QREKRAVGIGALFLGFLG spans the junction of the HIV major envelope glycoprotein gp120 and the transmembrane glycoprotein gp41 (amino acids 514-531). This peptide shows a slight degree of homology with ISP. Another immunosuppressive peptide (designated SUP1) having the amino acid sequence LQARILAVERYLKDQQL was derived from the gp41 region of HIV (amino acids 583-599) and has no significant homology to ISP. Both SUP1 and SUP2, when conjugated to KLH, suppress T-cell proliferation in response to mitogen stimulation. Unexpectedly, however, SUP2 is also immunosuppressive unconjugated.

Another immunosuppressive peptide (designated 34.1 and having the amino acid sequence AVERYLKDQQLLGIWGCSGKLIC) is derived from gp41 and has no homology to ISP or SUP2 but overlaps the SUP1 sequence. 34.1 has also been shown to be immunosuppressive in vitro when conjugated to KLH or when used unconjugated. Applicants maintain that these peptides are useful as therapeutic immunosuppressants and vaccines to prevent infection with HIV. Antibodies (monoclonal or polyclonal) to these HIV immunosuppressive peptides are also expected to have diagnostic and therapeutic potential in AIDS.

The peptides, polypeptides, proteins, compounds and methods

of the present invention will be better understood by reference to the following experiments and examples, which are provided for purposes of illusration and are not to be construed as in any way limiting the scope of the invention, which is defined by the claims appended hereto.

FIRST SET OF EXPERIMENTS

EXAMPLE 1 - Peptide-keyhole limpet hemocyanin (KLH) conjugate preparation

Peptides were synthesized by conventional solid-phase methods and crosslinked (conjugated) to KLH in the following manner. Peptide (5mg/ml in water) and KLH (5 mg/ml in water) were mixed together. Glutaraldehyde was added to this mixture to a final concentration of 0.04% (v/v). The reaction mixture was stirred for 30 minutes at room temperature and then dialyzed overnight against phosphate buffered saline (PBS) at 4°C.

EXAMPLE 2 - Antibody generation and purification

New Zealand White rabbits were injected with 200 µg of conjugate in 0.50 ml PBS and 0.50 ml Complete Freund's Adjuvant. Two booster injections of 200 µg conjugate/PBS in Freund's Incomplete Adjuvant were administered at four week intervals. Weekly serum samples were monitored for antibody titers to immobilized synthetic peptide by ELISA. Serum antibody samples found to recognize synthetic peptide were then tested for recognition of intact antigen by immunoblot analysis pursuant to the method of Example 3 below. In this test, extracts of FLV-infected cells (the cell line FL74) were resolved by SDS slab gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. After blocking with a Tris

buffered solution containing 3% gelatin, the membrane was sliced vertically into identical strips. These strips of resolved cellular proteins were probed with antisera to the p15E synthetic peptide and peroxidase-labelled anti-rabbit Ig diluted in Tris buffered saline containing 1% gelatin. Final visualization of antibody reactivity was obtained by incubating strips in an enzyme substrate solution of 4-chloro-1-naphthol. All serum samples that strongly recognized viral p15E were pooled and again titered by ELISA against peptide and by immunoblot analysis against intact (p15E) antigen. In all immunoblot studies, non-specific antibody staining was readily distinguished from specific antigen detection by probing identical blots with antiserum blocked with unlinked peptide.

Immunocytochemical detection of antigen, as defined by peptide blocking experiments, required affinity purification of antibody. Rabbit anti-peptide antibody was purified by passing antiserum through a CM-Affigel Blue (Bio-Rad) column to obtain an enriched Ig fraction. This Ig fraction was applied to a peptide affinity column prepared with epoxy-activated Sepharose 4B. The column was washed with phosphate buffer/0.5M KCl and antibody eluted with 1M acetic acid into tubes containing 3M phosphate buffer, pH 8. Active fractions were pooled, dialyzed and concentrated by ultrafiltration. All steps of antibody enrichment were monitored by peptide ELISA. The immunocytochemistry procedure used gold labelled second antibody and silver enhancement as described in European patent publication 158,746.

EXAMPLE 3 - Sample Preparation for Immunoblot Analysis

Cells were prepared for immunoblot analysis by first rinsing in PBS and then lyophilizing. The resulting cell

powder was suspended (6.0 mg/ml) in SDS sample buffer and further denatured in rapidly boiling water. The cell lysate was clarified (30,000 rpm; Beckman 50Ti rotor) at room temperature. Extracts from 200 µg were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 3% stacking/12% resolving gel. Proteins were electrophoretically transferred to nitrocellulose membranes in 192 mM glycine/20% methanol/10 mM Tris, pH 8.3 at 100 volts for 3 hours and 40 volts overnight.

EXAMPLE 4 - Comparison of antiserum detection of antigen in extracts of various tumor cell lines by Western blot analysis.

Rabbit anti-sera to a non-suppressive feline p15E peptide and ISP were immunoblot tested for detection of antigen in virus-infected cell extracts. Goat polyclonal antisera to intact murine p30gag and gp70env were included to confirm the presence of viral proteins in FL74 (FLV-infected lymphoma derived cell line) and NRK206-2/IC (murine leukemia/sarcoma virus [MLV/SV] infected) cells. Both antisera to p15E synthetic peptides detected Pr85env, p15E and p12E (proteolytic processed p15E) in FL74 cells, but not the MLV/SV or adult T cell leukemia viral (HTLV 1) homologs. The anti-ISP serum detected a 35,000 dalton protein (p35) in extracts of HTLV 1 infected cells (HUT102) but not the other two cell lines.

Anti-ISP serum was used to immunoblot extracts of normal PBLs and various cell lines derived primarily from human acute leukemias (Table 1). Every leukemia cell line examined, but not normal PBLs, expressed the p35 protein. Antiserum reactivity with antigen was blocked in all cell lines by soluble unconjugated peptide. The highest level of 35K protein expression was detected in the Raji cell

line.

TABLE 1
Anti-ISP Serum Detection of Cellular Antigen*

Cells	Immunoblot p35	Immunocyto-chemistry
FL74	Pr85env, p15E	+++
Raji	+	+++
CEM	+	+++
HUT102	+	+++
HL60	+	+++
K562	+	+++
EM2	+	+++
Human PBLs	-	+
A4318	+	++
NIH/3T3	Mr33,000	+

*Antiserum detection of antigen blocked with unlinked peptide; Thus a (-) indicates no antigen is detected; (+) = weak; (++) = moderate; and (+++) = strong.

Exposure to C kinase activators induces a change in expression of the activated oncoprotein P210bcr-abl (Kloetzer, et al. unpublished results). Immunoblot and immunocytochemical comparison of induced versus uninduced cells established a direct correlation between expression of P210bcr-abl and p35 (Table 2). For this reason, applicants maintain that expression of the p15E-related protein is a differentiation regulated event. It has not yet been determined whether variations in the amount of p35 detection are the result of altered rates of synthesis or altered processing of an undetected precursor protein.

TABLE 2
PMA Exposure Altered Expression of 35K Protein

Cells	10nM PMA	P210bcr-abl	p35
K562	none	+++	+
K562	5 days	-	-
EM2	none	+	+
EM2	5 days	+++	++

P210bcr-abl levels were determined by in vitro kinase assay. Detection of P210bcr-abl and 35K protein were estimated as none (-), weak (+), moderate (++) or strong (+++).

EXAMPLE 5 - Definition of the major epitope recognized by anti-ISP serum

Anti-ISP serum specifically recognized Pr85env, p15E and p12E in FLV-infected cells, and a 35,000 dalton cellular antigen in all human leukemia cell lines examined. The specificity of the anti-ISP serum for inact protein was tested by blocking the antiserum with homologous, i.e., similar but not identical to, peptides synthesized from murine, human, and bovine leukemia viral env sequences (Table 3). Overlapping feline p15E peptides were also evaluated for their ability to inhibit antibody detection of intact antigen. Results of these experiments show that antibody bound to the SDS-denatured protein is directed primarily against the variable portion of the feline ISP.

TABLE 3
Peptide Blocking of Anti-ISP Serum Recognition of Viral (FL74) and Cellular (Raji) Antigens*

Blocking Sequence	Pr85env/p15E*	p35*
(none)	+	+
QEGGLCAALKEEC	+	+
LDILFLQEGGLCAALK	-	-
(ISP) LQNRRGLDILFLQEGGLC	-	-
EVVLQNRRGLDILFL	+	+
KSLTSLSEVVLQNRRG	+	+
(MLV) LQNRRGLDLLFLKEGGLC	-	-
(HTLV1/2) AQNRRGLDLLFWEQGGLC	+	+
(BLV) AQNRRGLDWLYIRIGFQS	+	+
YQNRLALDYLLAAEGGVC (endogenous human virus)		

*Detection of antigen was evaluated as positive (+) or negative (-); endogenous human virus sequence was not tested

The ISP domain of viral p15E is the most highly conserved peptide domain among RNA tumor viruses. This conservation is shown below (Table 4) in a computer generated alignment of the predicted amino acid sequences (from the sequenced nucleotides) for FLV and HTLV 1 envelope domains (: = identical matches):

TABLE 4

```
                 |----------C18B---------| . |-------ISP--------|
FLV       510          520         530          540         550         560
FRQLQMAMHT DIQALEESIS ALEKSLTSLS EVVLQNRRGL DILFLQEGGL CAALKEECCF YADHTG
    :      ::  :   :       :  :        :::::: :  ::    ::: :  ::  :  :  :
GKSLLHEVDK DISQLTQAIV KNHKNLLKIA QYAAQNRRGL DLLFWEQGGL CKALQEQCRF PNITNS
HTLV 1 352        362        372        382        392        402
```

The only region of significant homology centers about the ISP peptide and a neutralizing antibody (e.g., antibody blocks in vitro virus infection) inducing peptide identified by Dr. John Elder (Scripps Institute, personal communication) called C18B. If the computer alignment is performed with the exception that amino acids of similar hydropathicities are also scored as "matches", the following alignment is achieved (Table 5):

Table 5

Identical matches plus the following equivalents:

Neutral or weakly hydrophilic: A = G = P = S = T
Hydrophilic with small side groups: D = N = E = Q
Hydrophilic with large side groups: R = H = K
Hydrophobic with small side groups: I = L = M = V
Hydrophobic with large side groups: F = W = Y

```
                  |-------ISP---------|
FLV    530        |   540         550 |       560
ALEKSLTSLS EVVLQNRRGL DILFLQEGGL CAALKEECCF YADHTGLV
   * *  ** *   ****** **** ***** * ** *** *       * *
KNHKNLLKIA QYAAQNRRGL DLLFWEQGGL CKALQEQCRF PNITNSHV
HTLV 1 372         382         392         402
```

It is apparent that even though exact sequence homology is not retained in the C terminal halves of ISP domains, the hydrophilic properties are highly conserved. For this reason, applicants contemplate that the C terminal portion, and possible amino acid sequences extending to FLV amino acid 560, play an important role in p15E immunosuppressive activity.

Overall sequence homology between FLV and HTLV 1 or 2 envelope sequences are slight but quite distinct in the ISP

domains. We have also identified a very distantly related sequence homology between FLV and the human immunodeficiency viral (HIV) envelope gene products (Table 6):

TABLE 6

```
                    <------"ISP"-------->
FLV p15E  56-103    63   SLSEVV LQNRRGLDILFLQEGGLC AALKEECCFYADHT  100
                         ::   : :  : :  ::        :
                         *   **  ***** *  *  * *  **        *
HIV  I    env       501   AKRRVV QREKRAVGIGALFLGFLG AAGSTMGAASMTLT  538
                              <-----"SUP-2"------->
```

: indicates identical matches
* indicates identical matches plus the following equivalents:

A = G = P = S = T
F = W = Y
I = L = M = V
Q = D = E = N
K = H = R

EXAMPLE 6 - Antibody blocking of immunosuppression attributed to viral p15E and p15E-like proteins.

Persistent infection by feline leukemia virus is frequently associated with non-regenerative aplastic anemia and opportunistic infections resulting from suppressed cell-mediated immunity. These clinical features are attributed to the direct effects of the viral p15E. A biologically active synthetic peptide selected from the murine p15E amino acid sequence has been shown by others to exhibit in vitro effects similar to the intact viral protein. Applicants have confirmed that a synthetic peptide from the homologous region of feline viral p15E has similar in vitro effects. Applicants have also discovered that rabbit polyclonal antiserum to ISP:KLH conjugate specifically recognizes, as detected by peptide blocking of antibody, feline viral p15E and a cross-reacting antigen in many human leukemia cells. Peptide blocking experiments with overlapping sequences have indicated that the major epitope to which the antibody is directed lies within the carboxyl terminal half of the ISP sequence. Monoclonal antibodies to ISP developed using the basic methods of Kohler and Milstein (Nature 225, 1975) may be screened for recognition of available homologous and overlapping peptides (Table 3). An alternative approach is to generate monoclonal antibodies to purified feline p15E and screen hybridoma clones for ELISA recognition of ISP. The unique advantage of this approach is the generation of monoclonal antibodies against the biologically active site on native protein. After preliminary selection of clones for recognition of antigen, all antibodies may be screened for the ability to block virus-mediated inhibition of mitogen-treated spleen cells. A monoclonal antibody that blocks the suppressive activity of viral p15E by binding to the active site may be tested for the ability to ameliorate

symptoms of virus induced disease. Similar testing of effects on p15E-like proteins detected in various human cancers may also be tested as a passive immunotherapy reagent or as means of targeting other drugs to cancer cells.

EXAMPLE 7 - Synthetic peptide as an immunosuppressant therapeutic agent

Synthetic or recombinant (DNA)-derived peptides with amino acid sequences derived from the conserved domain of viral p15E (entire FLV domain shown in Table 5) or p15E-like proteins can serve as immunosuppressive agents. Such peptides are useful for suppressing the immune system after tissue or organ grafts. In addition, biologically active peptides may provide therapeutic benefits to patients with autoimmune diseases.

EXAMPLE 8 - Synthetic peptide as a retrovirus vaccine

The feline ISP:KLH conjugate was used to immunize four cats. Two injections of 200 µg conjugate in 0.5ml DPBS plus 1.0 mg 7-methyl-8-oxoguanosine (immune stimulator) in 0.5 ml Montinide/Draekol were administered 14 weeks apart. Similar injections of a neutralizing antibody inducing peptide, called I85B, from the gp70 region of the FLV envelope protein were given to two cats. At week 28 following the last injection, the six peptide vaccinated cats and six untreated cats were immunosuppressed with glucocorticoids and challenged with live Rickard strain of feline leukemia virus. Signs of infection were monitored before and after virus challenge by ELISA using monoclonal antibody to the major viral core antigen (p27) as the capture reagent and peroxidase-conjugated immune globulin G (LeukAssay-F available from Pitman-Moore, N.J.). Five

weeks after challenge, both cats immunized with I85B:KLH were antigenemic (p27 detected in the serum). Only one of the four cats vaccinated with ISP:KLH displayed signs of virus in the serum. Four of six cats in a control group (no vaccination) became antigenemic after challenge. Thus, applicants have shown the first indication that immunization with ISP-conjugate protected cats against FLV infection. Similarly, immunization of a subject with the appropriate viral p15E peptide-conjugate (Table 3) will protect the subject from subsequent viral infection.

TABLE 7

Suppressive Activity of Feline Leukemia Virus ISP Overlapping Peptide-Conjugates as measured by inhibition of ConA-induced T-cell proliferation

Peptide Sequence	Suppressive Activity	Peptide Code
KSLTSLSEVVLQN.....	+	c93
KSLTSLSEVVLQNRRG.....	++	c94
(C)SLSEVVLQNRRGLDI.....	-/+	c95
(C)EVVLQNRRGLDILFL.....	-/+	c96
(C)LQNRRGLDILFLQEG.....	-/+	c97
RRGLDILFLQEGGLC.....	+	c98
LDILFLQEGGLCAALK.....	-/+	c99
LFLQEGGLCAALK.....	-	c100
QEGGLCAALKEEC.....	++	c101
GLCAALKEECCFLK.....	-	c102
AALKEECCFLKEEC.....	+++	c103
LQNRRGLDILFLQEGGLC.....	+++	(ISP)

(-) = no activity, (-/+) = slight activity, (+) = fair activity, (++) = good activity, (+++) = very good activity; (C) = cysteine not in predicted amino acid sequence but added for cross-linking purposes in other studies.

EXAMPLE 9 - HIV peptides with immunosuppressive activity

Peptide sequences derived from the HIV sequences have been synthesized using standard solid phase peptide technology and have been tested for the ability to suppress mitogen-induced proliferation of mouse T-cells (Table 8). Applicants contemplate that those peptides observed to have suppressive activity are useful as a vaccine against Acquired Immunodeficiency Syndrome.

TABLE 8

Immunosuppression Mediated by HIV Peptides

PEPTIDE	SEQUENCE	INHIBITION OF T-CELL PROLIFERATION
HIV peptides:		
C232	QLTVWGIKQLQARIL	+
C233	GIKQLQARILAVERY	-
4B	QLQARILAVERYL	-
SUP1	LQARILAVERYLKDQQL	-
SUP1-KLH		++
C234	QARILAVERYLKDQQ	-
US1	RILAVERYLKDQQLLGIWGCS	-
C235	AVERYLKDQQLLGIW	-
E34.1	AVERYLKDQQLLGIWGCSGKLIC	-
E34.1-KLH		++++
E34.2	LKDQQLLGIWGCSGKLIC	-
SUP2	QREKRAVGIGALFLGFLG	++
SUP2-KLH		+++
C92	TKAKRRVVQREKRA	-
C221	AVEVGIGALFLGFLGAA	-
FLV peptide:		
ISP	LQNRRGLDILFLQEGGLC	-
ISP-KLH		+++
KLH-glutaraldehyde control		-

*KLH - Peptide coupled to KLH via glutaraldehyde.
(-) = non-existent; (+) = weak; (++) = moderate;
(+++) = strong; (++++) = very strong.

The assay performed was Concanavalin A-induced T cell proliferation of murine (C57B1/6 strain) spleen cells utilizing the procedure set forth in Example 10.

EXAMPLE 10 - Assay for immunosuppression - Concanavalin A-induced T-cell proliferation of murine (C57BL/6 strain) spleen cells

1. A spleen cell supspension was prepared from C57BL/6-strain mice using asceptic technique.

2. The spleen cell suspension was applied onto a pre-washed and pre-warmed (37°C) nylon-wool column (ca. 10^8 cells/0.6 gr. nylon-wool). The cells were incubated for 45 minutes and eluted dropwise with warmed medium. An enriched T cell population was collected.

3. Collected cells (10^5/well) were added to sterile, 96-well microtiter plates in which various concentrations of peptide (400-25 micrograms/ml) were previously added. The plates were then incubated for 30 mintues at 37°C in 5% CO_2.

4. Concanavalin A (Con A) mitogen was then added (1 microgram/well) to respective wells in the plate. Control wells included cells with and without Con A. Final volumes in each well were 200 microliters. the following exemplifies a typical protocol culture set up:

Spleen cells:	50 µl/well (2 X 10^6/ml)
Peptide dilution:	100 µl/well
Mitogen (Con A):	50 µl/well (4 µg/ml)

5. The plates were then incubated for 3 days at 37°C in 5%

CO_2. Tritiated (3H) thymidine (1 microCurie/well) was added to each well 16 hours before harvesting.

6. The ^{3}H-thymidine-pulsed plates were then harvested using a PHD cell harvester. The harvester filter discs were placed in individual scintillation vials, scintillation fluid added, and the vials counted for presence of radioisotope.

7. Proliferation was determined as counts per minute (CPM) or as a percent of control proliferation (i.e., CPM of experimental/CPM of Con A control X 100).

Medium: RPMI 1640 medium
L-glutamine (2 mM)
Sodium pyruvate (1 mM)
Gentamycin sulfate solution (20 µg/ml)
Selected heat-inactivated fetal bovine serum (5%)

SECOND SET OF EXPERIMENTS

Immunosuppressive Assay: The basic proliferation assay was performed in 96-well microtiter plates and involved stimulating murine spleen cells ($1x10^5$ cells/well) with a mitogen, e.g., Concanavalin A (Con A) for T cells and lipopolysaccaride (LPS) for B cell, which induces lymphoid cells to become activated and proliferate in culture. The lymphoid cell cultures were incubated for 3-7 days, depending on the experiment, and the cultures were pulsed for the last 12-24 hours with the radio-labeled nucleotide ^{3}H-thymidine, which is incorporated into the deoxyribonucleic acid (DNA) of the proliferating cells. The cells from individual culture wells were harvested onto glass-fiber discs using a multichannel cell harvester and the individual discs, placed in fluor-containing

scintillation fluid, and analyzed in a scintillation spectrophotometer for the presence of radioactivity. The level of cell activation, i.e., proliferation, was measured as the amount of DNA-incorporated ^{3}H-thymidine detected in mitogen-stimulated cultures relative to that of non-stimulated control cultures, i.e., cultures with cells alone. The data was recorded as the stimulation index (S.I.) (calculated as experimental counts per minute (CPM)/control culture CPM) or as a percentage of control proliferation, i.e., experimental CPM/positive control cultures (cells + mitogen) X 100.

In assays designed to determine the immunosuppressive activity of peptides, cultures were prepared as described above except that the suppressive peptide, at varying concentrations, was mixed into the initial cultures. Suppression of cell proliferation was interpreted as the diminution of the S.I. or percent (%) control proliferation in cultures containing suppressive peptide compared to cultures not exposed to the peptide. Suppression was considered significant if greater than 40%. These experiments were not restricted to any particular mouse strain.

<u>δI6B:ISP Mediated Supression:</u> Balb/c spleen cells were stimulated with Con A (4 micrograms/ml) in the presence of titrated amounts of δI6B:ISP and the level of ^{3}H-thymidine incorporation was determined on day 4. δI6B:ISP induced significant suppression of T cell proliferation (60%) at a concentration of 12.5 µg/ml (Fig. 1). Interestingly, higher concentrations of peptide, e.g., 100 µg/ml, did not cause suppression. These results were reproducible in additional experiments. Upon examination of these cultures, cell numbers and viability were comparable in both control and peptide-treated control

wells. This indicates that the suppression was not the result of peptide toxicity in culture.

Further experiments were performed to examine the relative abilities of the δI6B, ISP, and δI6B:ISP peptides to suppress T cells. Cells were stimulated with mitogen and cultured for 4 days in the presence of the respective peptides. The δI6B:ISP peptide induced significant suppression, i.e., 78%, compared to either δI6B (35%) or ISP (28%) (Fig. 2). It was noted that the ISP peptide induced suppression only when conjugated to KLH, whereas δI6B:ISP was active as a free peptide. Furthermore, the ISP-KLH conjugate consistently induced suppression at concentrations of 100-200 μg/ml (data not shown).

In related studies, peptides were synthesized which are extensions of the ISP peptide in the context of the native viral sequence as well as an analog form. These peptides were examined in order to determine whether a longer peptide would be suppressive without requiring conjugation to a carrier or if suppressive activity could be improved. These extension peptide sequences were LQNRRGLDILFLQEGGLCAALKECCFYADH and LQNRRGLDILFLQEGGLCAALKECCFLKEE.

BALB/c mouse spleen cells were cultured for 4 days in the presence of mitogen (Con A) and varying concentrations of the two extension peptides. Neither peptide exhibited any suppressive activity in the murine T cell proliferation assays.

Specificity of Suppression: Experiments were performed to determine the specificity of the δI6B:ISP-mediated suppression of cell proliferation and the nature of the cell type being suppressed. Spleen cell cultures were

stimulated with Con A or lipopolysaccharide in the presence of δI6B:ISP. The cultures were assayed on days 4 and 5. The δI6B:ISP peptide was able to suppress T cell proliferation (Fig. 3A) but not B cell proliferation (Fig. 3B).

In additional experiments, the effect of δI6B:ISP on IgG secretion by JY cells, a human B lymphoblastoid cell line, was examined. The levels of secreted IgG were comparable between untreated and treated cell cultures, indicating that δI6B:ISP does not suppress antibody production.

Time Kinetics of In Vitro Supression: During the process of examining δI6B:ISP-induced suppression, applicants observed in a series of experiments with murine and human cells that the peptide did not suppress proliferation. It was noted that the assays had been incubated for 3 days rather than 4 or 5 days and the fact that mitogen-induced proliferation is generally signficant after 3 days of culture. Therefore, an experiment was performed to determine the influence of in vitro culture time on the ability of δI6B:ISP to suppress T cell proliferation. The results dramatically showed that suppression is not observed at day 3 of culture, but only occurs after day 4 (Fig. 4). This indicates that the peptide interacts with a metabolic pathway and requires a certain amount of time to manifest this effect.

It has also been found that pokeweed mitogen (PWM)-stimulated human spleen cell proliferation is suppressed by δI6B:ISP.

Recognition of δI6B:ISP by Anti-Peptide Sera: Applicants examined the ability of anti-I6B serum (R112-5) and affinity-purified rabbit antiserum (AP558-28) against the

ISP peptide, as well a monoclonal antibody (9-14F2-3) which reacts with ISP, to bind δI6B:ISP in order to determine whether the respective antibody-binding sites remained intact after combining the δI6B and ISP sequences. A control affinity-purified rabbit antibody (AP67) against the c-abl oncogene was also used. The results obtained indicate that both the anti-I6B and anti-ISP antibodies react with δI6B:ISP (Fig. 5).

THIRD SET OF EXPERIMENTS

Protein sequence alignment methods: Identification of proteins with sequences related to the selected polypeptides was accomplished using the SCANSIM program (verison 3.02) of PC Gene (release 4.05 from Intelligenetics, Inc.) and the Swiss Protein Database (release 2). Application of the Needleman-Wunsch algorithm (PEP/ALIGN), for a more rigorous alignment of selected sequences, was accessed via Bionet from Intelligenetics, Inc.

Polypeptide sequences: Polypeptides were selected from the predicted protein sequence of the FLV envelope gene precursor (Elder et al., J. Virology 46: 871-880, 1983) and were synthesized by conventional solid phase methods.

Generation of antisera to synthetic polypeptides: Synthetic polypeptides were chemically coupled to keyhole limpet hemocyanin (KLH) at 1:1 (w/w) ratios by mixing 30 min. at room temp. in 0.04% glutaraldehyde. New Zealand White rabbits were injected with 200 µg of dialyzed conjugate in Complete Freund's Adjuvant followed by two or more booster injections of 200 µg conjugate in Incomplete Freund's Adjuvant.

Protein labeling by metabolic incorporation of [^{35}S]amino acids: For each immunoprecipitation, 2 x 10^7 cells were metabolically labeled with 0.5 mCi [^{35}S]methionine (or a [^{35}S]met/[^{35}S]cys mixture) in 2.0 ml methionine-deficient RPMI 1640 supplemented with L-lysine, L-leucine, L-glutamine and 5% dialyzed calf serum.

Detection of [^{35}S]met-labeled antigen by immunoprecipitation: 2 X 10^7 labeled cells were lysed in 1.0 ml lysis buffer (0.05% SDS, 1% Triton X-100, 1% deoxycholate, 1 mM EDTA, 150 mM NaCl, 100 KIU Trasylol/ml, 50 mM MOPS, pH 7.0) and clarified by centrifugation for 10 min. at 10,000 rpm (Sorvall SS34 rotor).

Antigen was precipitated by addition of 0.020 ml antiserum for 18 hrs. on ice. Immune complexes were absorbed by incubation with 0.100 ml Sepharose: Protein A (Pharmacia) for 60 min. on ice. The Sepharose-immobilized immune complex was washed with cell lysis buffer, denatured by addition of 0.050 ml SDS sample buffer (2% SDS, 10% glycerol, 0.125 M Tris-HCl, pH 6.8), and immersed for 3 minutes in rapidly boiling water. The beads were pelleted by centrifugation, the supernatant fluid was diluted 20 times in cell lysis buffer, and the immunoprecipitation steps were repeated. The resulting immune complex was dissociated with 2% SDS sample buffer containing 10% 2-mercaptoethanol. Samples were resolved by SDS-polyacrylamide gel electrophoresis using 3% stacking gels and either single concentration (12%) or gradient (10-20%) resolving gels (Laemmli, Nature 227: 680-685, 1970). Radiolabeled antigen was detected by conventional fluorographic methods.

Immunocytochemical detection of antigen: Rabbit antibody to synthetic peptide was purified in two steps. An

immunoglobulin fraction was collected from serum by 25% to 50% (w/v) ammonium sulfate precipitation. After dialysis against phosphate buffered saline, the sample was applied to a peptide affinity column prepared with epoxy-activated Sepharose 4B (Pharmacia). The column was washed with phosphate buffer/0.5M KCl and antibody was eluted using 1 M acetic acid into tubes containing 3 M phosphate buffer, pH 8. Active fractions were pooled, dialyzed and concentrated by ultrafiltration. The immunocytochemistry procedure used gold-labeled second antibody and subsequent silver enhancement described in Eureopean patent publication 158,746.

Identification of proteins containing sequences homologous to ISP

The ISP domain of FLV p15E is highly conserved among RNA tumor viruses. A SCANSIM program search of the entire Swiss Protein database (release 2) identified nine viral envelope sequences with peptide domains very similar to FLV-ISP. The common sequence "QNRRGLDXL" at the carboxyl terminus of all nine env peptides was used to search the entire Protein database for related sequences. Table 9 below summarizes results of a search for QNRRGLDXL-containing sequences in a subset of the Swiss Protein database selected with the keywords "interferon", "interleukin" and "tumor necrosis factor". Similar results (not shown) were obtained using the PEP/SEARCH/HOMOLOGY program (Intelligenetics) on an equivalent subset of the Protein Information Resources (PIR; Intelligenetics).

TABLE 9

Interferon/Interleukin/Tumor Necrosis Factor Alignments with the Highly Conserved Portion of Viral ISP

(Q N R R G L D X L)

Position From	To	Sequence	Description
33 to	41	R N R R A L I L L	human IFN α-10 precursor
33 to	41	R N K R A L K V L	mouse IFN α-2 precursor
33 to	41	R N K R A L T L L	mouse IFN α-1 precursor
33 to	41	D N R R T L M L L	human IFN α-1 precursor
33 to	41	G N R R A L I L L	human IFN α-4 precursor
33 to	41	G N R R A L I L L	human IFN α-5 precursor
33 to	41	G N R R A L I L L	human IFN α-6 precursor
33 to	41	G N R R A L I L L	human IFN α-9 precursor
40 to	48	V N P R S L E K L	human IFN τ induced protein precursor
33 to	41	N N R R T L M L M	human IFN α-8 precursor
33 to	41	G S R R T L M L L	human IFN α-2 precursor
10 to	18	G S R R T L M L L	human IFN α-3 precursor
128 to	136	V Q R K A I H E L	human IFN τ precursor
30 to	38	Q Q R R S L A L C	bovine IFN β-2 precursor
110 to	118	K K R D D F E K L	human IFN τ precursor
114 to	122	Q Q L N D L E V L	human IFN α-4 precursor
126 to	134	V Q R Q A F N E L	mouse IFN τ precursor
105 to	113	L N R R A N A L L	human TNF precursor
72 to	80	Q K T Q A I S V L	human IFN α-9 precursor
72 to	80	Q K T Q A I S V L	human IFN α-10 precursor
127 to	135	I Q H K A V N E L	rat IFN τ precursor
126 to	134	E E R V G E T P L	human IFN α-1 precursor
72 to	80	Q K A Q A I S V L	human IFN α-4 precursor
72 to	80	Q K A Q A I S V L	human IFN α-5 precursor
72 to	80	Q K A Q A I S V L	human IFN α-6 precursor
16 to	24	Q K A Q A I S V L	human IFN α-7 precursor
72 to	80	Q K A Q A I S V L	human IFN α-8 precursor
42 to	50	V Q M R R L S P L	mouse IFN α-1 precursor
77 to	85	P E S K A I K N L	human IFN τ induced protein precursor
111 to	119	D L H Q Q L N D L	mouse IFN α-1 precursor
42 to	50	A Q M R R L P F L	mouse IFN α-2 precursor
111 to	119	D L H Q Q L N D L	mouse IFN α-2 precursor

Application of a more rigorous alignment program (Needleham/Wunsch [PEP/SEARCH/ALIGN]) shows regional similarities between the ISP domain of viral p15E and the amino terminal amino acids 21-47 of the human alpha interferon sequence. A recent study using oligonucleotide site-directed mutagenesis has shown that the receptor binding site of human alpha interferon lies within the amino terminal 10-44 amino acids (Shafferman, et al., J. Biol. Chem. 262: 6227-6237, 1987). Applicants contemplate that biologically-active ISP and ISP-related antigens mediate some of their effects through cellular receptors for alpha interferon.

ISP-related antigens are released into the growth medium of divese human leukemia cell lines

Four leukemia derived cell lines were metabolically labeled with [^{35}S]met for 4 to 6 hours and "chased" 40 hours with complete growth medium:

Cell Line	Derivation
HL60	human Acute Myelogenous Leukemia
Raji	human Burkitt's lymphoma
K562	human Ph'$^{+}$ Chronic Myelogenous Leukemia erythroleukemic blast crisis
FL74	FLV^{+} feline lymphoma

The conditioned growth medium was clarified by low and high speed centrifugation. Antigen was then immunoprecipitated from the resulting supernatant fractions. The purified antigens were separated on 10-20% gradient SDS-gels and detected by fluorography. A soluble 110K antigen was detected in the conditioned growth medium of all leukemia cell lines tested including the FLV^{+} FL74 cells.

Overlapping FLV ISP peptides block FLV ISP antiserum recognition of the 110,000 dalton antigen from Raji cells

The effects of overlapping FLV ISP peptides were compared for their ability to block FLV ISP antiserum detection of [^{35}S]met-labeled FLV gPr85^{env}/p15E and Raji cell antigens. The results summarized in Table 10 show that overlapping FLV peptides display very similar abilities to block detection of feline viral and human leukemia cell derived antigens.

TABLE 10

FLV Peptide Blocking of FLV-ISP Antiserum Recognition of Immunoprecitated FLV-p15E and Raji 110K Protein

		[^{35}S]met Antigen Detection	
Peptide	Sequence	FLV p15E	Raji 110K
none		++++	++
FLV ISP	LQNRRGLD ILFLQ EGGLC	-	-
F198	VVLQNRRGLD IL	++	+
F197	VLQNRRGLD ILF	+	-
F196	LQNRRGLD ILFL	+	-
F195	QNRRGLD ILFLQ	-	-
F194	NRRGLD ILFLQ E	-	-
F186	LD ILFLQ EGGLC	++++	++
F187	D ILFLQ EGGLCA	++++	++
F188	ILFLQ EGGLCAA	+	-
F189	LFLQ EGGLCAAL	++++	++
F190	FLQ EGGLCAALK	++++	++

(-) = no activity; (+) = weak; (++) = moderate; (++++) = very strong;

T cell mitogen (PHA) stimulates synthesis of [^{35}S]met-labeled 110K antigen in normal human PBLs

Histopaque enriched peripheral blood leukocytes from a normal, healthy donor were stimulated with 10 µg/ml phytohemagglutinin. After four days in culture, unstimulated and stimulated cells were labeled for 4 hours with [^{35}S]met and lysed for immunoprecipitation. The results indicate that very low levels of [^{35}S]met-labeled antigen were detected in unstimulated cells and high levels of antigen were detected in the mitogen stimulated cells.

The 110K antigen is detected in the human epidermoid carcinoma-derived cell line A431 but not in murine 3T3 fibroblasts

Immunocytochemistry results indicate that the 110K antigen can be weakly detected in the human cell line A431 cells but not in Swiss Albino 3T3 cells. An initial immunoprecipitation experiment was conducted with 4 hour [^{35}S]met-labeled cell extracts to determine if the 110K antigen is detected in either cell line. The 110K protein was clearly detected in A431 cells but not in 3T3 cells.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity or understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims. For example, the peptides of the present invention may be modified by substitution of conservative or non-conservative amino acids in the peptides. Likewise the subject peptides may be subject to various other changes, such as insertions or deletions of amino acids where such changes may provide for or have no substantial affect upon, the desired immunosuppressive activity *in vitro* or *in vivo*.

What is claimed is:

1. An immunosuppressive peptide which comprises a peptide having less than about 40 amino acid residues, said amino acid residues comprising an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being selected from amino acid sequences included within the amino acid sequence LQNRRGLDILFLQEGGLCAALKEECCF.

2. An immunosuppressive compound which comprises a peptide of claim 1 conjugated to a carrier molecule.

3. An immunosuppressive compound which comprises a peptide having less than about 40 amino acid residues, said amino acid residues comprising an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being selected from amino acid sequences included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
QREKRAVGIGALFLGFLG,
QLTVWGIKQLQARIL
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
AKLRERLKQRQQ,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,

LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ, and
LGSRRTLMLLAQMRKIS,

said peptide being conjugated to a carrier molecule.

4. A compound of claim 2 or 3 wherein the carrier molecule is keyhole limpet hemocyanin.

5. A compound of claim 2 or 3 wherein the carrier molecule is conjugated to the peptide with glutaraldehyde.

6. An immunosuppressive peptide which comprises a peptide having less than 40 amino acid residues, said amino acid residues comprising an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being selected from amino acid sequences included within the amino acid sequence QREKRAVGIGALFLGFLG or QLTVWGIKQLQARIL.

7. An immunosuppressive peptide which comprises an antigenic determinant homologous to an antigenic determinant of the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC.

8. An immunosuppressive peptide which comprises a first amino acid sequence of at least 5 amino acid residues, said first amino acid sequence being selected from amino acid sequences included within the amino acid sequence AKLRERLKQRQQ, and at least one other amino acid sequence of at least 5 amino acid residues, said other amino acid sequence being selected from amino acid sequences included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

9. An immunosuppressive peptide of claim 8 wherein the amino terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ is linked to the carboxy terminus of the amino acid sequence included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,

LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

10. An immunosuppressive peptide of claim 8 wherein the carboxy terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ is linked to the amino terminus of the amino acid sequence included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,

LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

11. An immunosuppressive peptide of claim 8 wherein the carboxy terminus and the amino terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ are each linked to an amino acid sequence included within an amino acid sequence selected from the group of amino acid sequences consisting of

LQNRRGLDILFLQEGGLC,
AALKEECCFLKEEC,
QEGGLCAALKEEC,
KSLTSLSEVVLQNRRG,
LQARILAVERYLKDQQL,
AVERYLKDQQLLGIWGCSGKLIC,
LQNRRGLDLLFLKERGLC,
AQNRRGLDLLFWEQGGLC,
LQNRRGLDLLTAEQGGIC,
AQNRRGLDWLYIRLGFQS,
LRNRRALILLAQMGRIS,
LDNRRTLMLLAQMSRIS,
LGNRRALILLAQMRRIS,
LGNRRALILLGQMGRIS,
LNNRRTLMLMAQMRRIS,
PVNPRSLEKLEIIPASQ,
LGSRRTLMLLAQMRKIS,
QREKRAVGIGALFLGFLG, and
QLTVWGIKQLQARIL.

12. An immunosuppressive peptide of claim 11, wherein the amino acid sequence linked to the carboxy terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ is the same as the amino acid sequence linked

to the amino terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ.

13. An immunosuppressive peptide of claim 11, wherein the amino acid sequence linked to the carboxy terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ is different from the amino acid sequence linked to the amino terminus of the amino acid sequence included within the amino acid sequence AKLRERLKQRQQ.

14. An immunosuppressive peptide of claim 8 which is cyclic.

15. A polymer which comprises repeating units of the peptide of claim 8.

16. A dimer which comprises two peptides of claim 8.

17. An immunosuppressive peptide which comprises the amino acid sequence AKLRERLKQRQQLQNRRGLDILFLQEGGLC.

18. An immunosuppressive peptide which comprises the amino acid sequence AKLRELKQRQQLQNRRGLDILFLQEGGLC.

19. A purified polypeptide which comprises an antigenic determinant for which an antibody generated against the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC has affinity, the polypeptide being expressed in mammalian cancer cells.

20. A purified polypeptide of claim 19 having an apparent molecular weight of about 110,000 daltons.

21. A purified polypeptide of claim 19 having an apparent molecular weight of about 35,000 daltons.

22. A purified nucleic acid sequence which encodes the polypeptide of claim 19.

23. A purified nucleic acid sequence which encodes the polypeptide of claim 20.

24. A purified nucleic acid sequence which encodes the polypeptide of claim 21.

25. An antibody which has affinity for the polypeptide of claim 19, 20, or 21.

26. A polyclonal antibody of claim 25.

27. A monoclonal antibody of claim 25.

28. A purified protein which has affinity for the polypeptide of claim 19 and which is present on the surface of hematopoietic cells.

29. A therapeutic composition which comprises the antibody of claim 25 and a pharmaceutically acceptable carrier.

30. A method for treating an immunologically or hematopoietically suppressed subject which comprises administering to the subject an effective, immunosuppressing blocking amount of the therapeutic composition of claim 29.

31. A therapeutic composition which comprises the purified polypeptide of claim 19, 20, or 21, or a peptide having an amino acid sequence of at least 5 amino acid residues, said amino acid sequence being included within the purified polypeptide, and a pharmaceutically acceptable carrier.

32. A method for suppressing the immune system of a subject which comprises administering to the subject an effective immunosuppressing amount of the therapeutic composition of claim 31.

33. A therapeutic composition which comprises a portion of the purified protein of claim 28 having immunosuppressive peptide-binding activity and a pharmaceutically acceptable carrier.

34. A method for treating an immunologically or hematopoietically suppressed subject which comprises administering to the subject an effective, immunosuppressing blocking amount of the therapeutic composition of claim 33.

35. A method for detecting a cancer cell in a sample which comprises detecting a cell from the sample which expresses a polypeptide having an antigenic determinant which reacts with an antibody raised to the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC, said polypeptide being expressed in mammalian cancer cells.

36. A method for diagnosing cancer in a subject which comprises detecting a polypeptide having an antigenic determinant which reacts with an antibody raised to the peptide having the amino acid sequence LQNRRGLDILFLQEGGLC, said polypeptide being expressed in mammalian cancer cells, or a portion of said polypeptide which includes said antigenic determinant, in a body fluid sample taken from the subject.

37. A vaccine which comprises the peptide of claim 1, 6, 7, 8, 17, or 18 and a pharmaceutically acceptable carrier.

38. A method for immunizing a subject against a retroviral infection which comprises administering to the subject an effective immunizing amount of the vaccine of claim 37.

39. A vaccine which comprises the compound of claim 2 or 3 and a pharmaceutically acceptable carrier.

40. A method for immunizing a subject against a retroviral infection which comprises administering to the subject an effective immunizing amount of the vaccine of claim 39.

41. A therapeutic composition which comprises the peptide of claim 1, 6, 7, 8, 17, or 18 and a pharmaceutically acceptable carrier.

42. A method for suppressing the immune system of a subject which comprises administering to the subject an effective immunosuppressing amount of the therapeutic composition of claim 41.

43. A therapeutic composition which comprises the compound of claim 2 or 3 and a pharmaceutically acceptable carrier.

44. A method for suppressing the immune system of a subject which comprises administering to the subject an effective immunosuppressing amount of the therapeutic composition of claim 43.

FIGURE 1

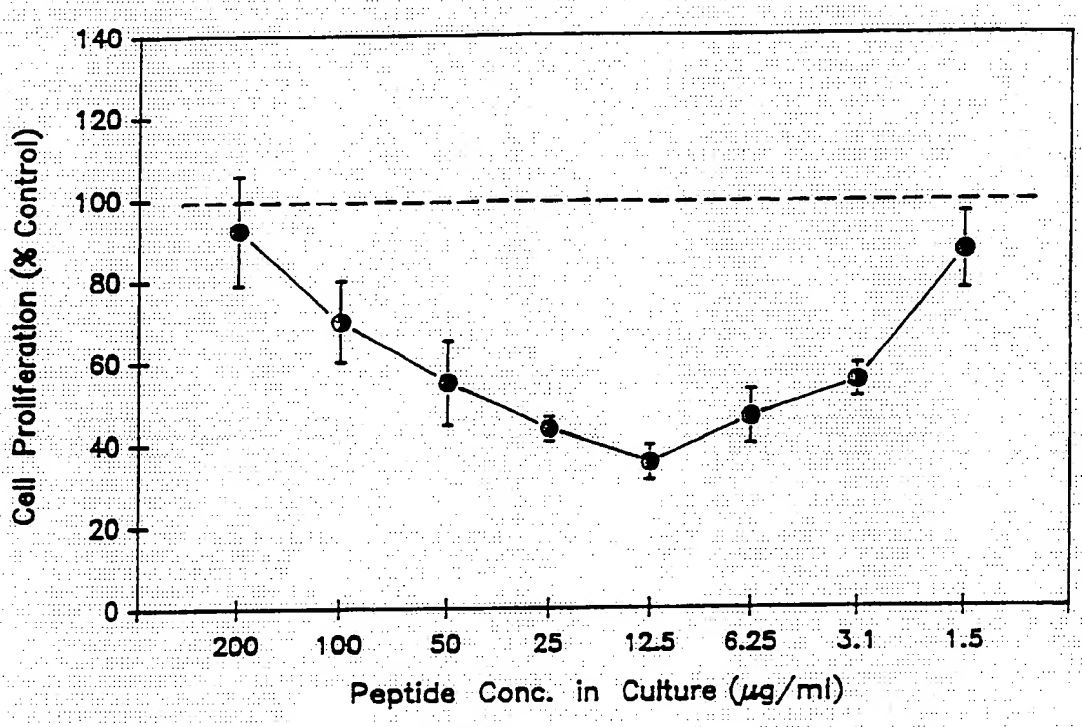

140
120
100
80
60
40
20
0
Cell Proliferation (% Control)
200
100
50
25
12.5
6.25
3.1
1.5
Peptide Conc. in Culture (μg/ml)

FIGURE 2

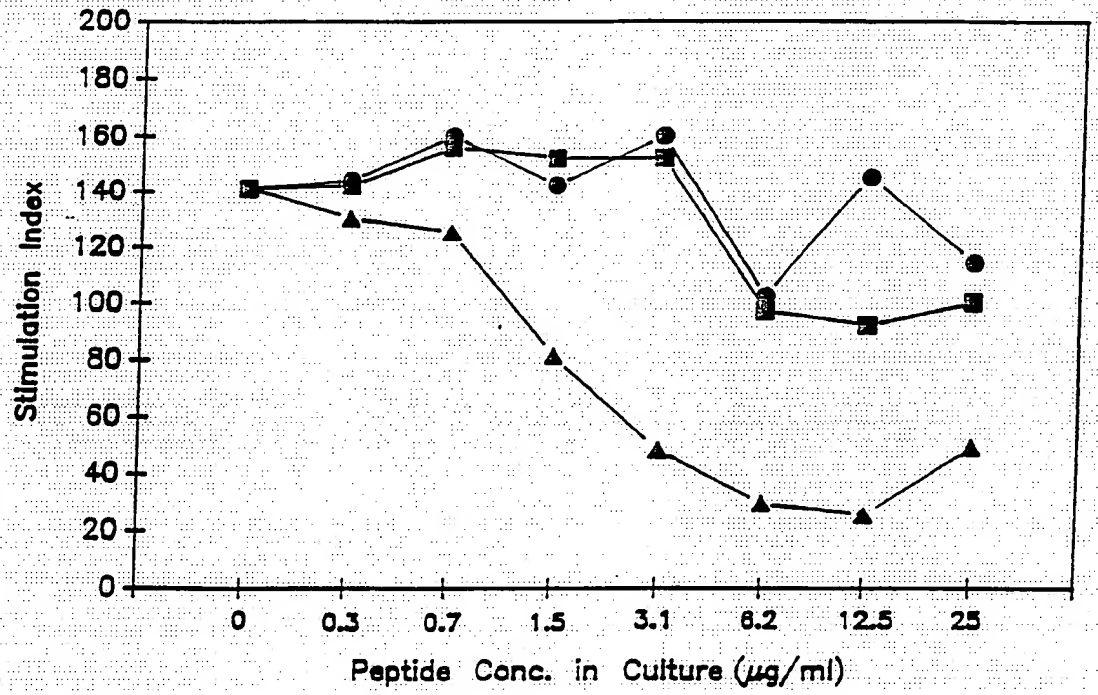

Stimulation Index
200
180
160
140
120
100
80
60
40
20
0
0
0.3
0.7
1.5
3.1
6.2
12.5
25
Peptide Conc. in Culture (μg/ml)

FIGURE 3A

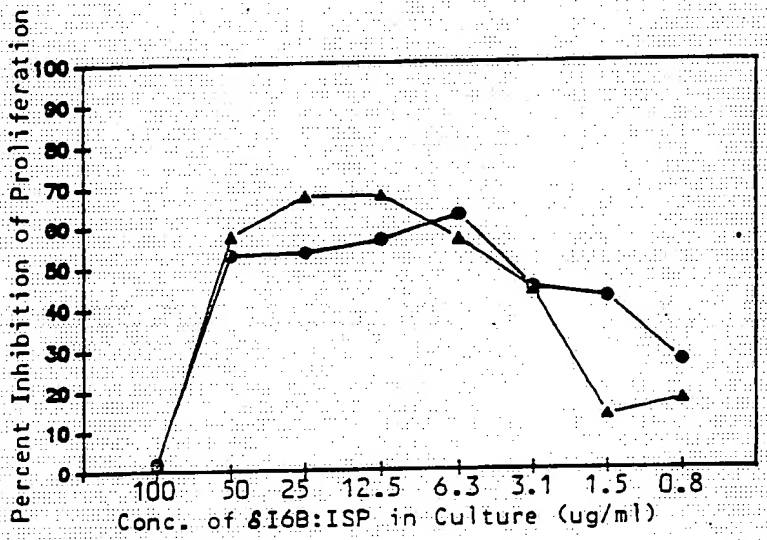

Percent Inhibition of Proliferation
100
90
80
70
60
50
40
30
20
10
0
100
50
25
12.5
6.3
3.1
1.5
0.8
Conc. of δI6B:ISP in Culture (ug/ml)

FIGURE 3B

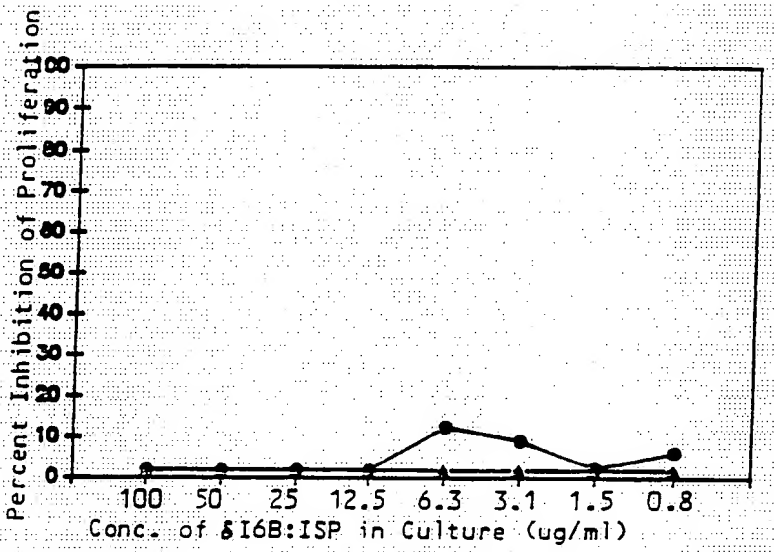

Percent Inhibition of Proliferation
100
90
80
70
60
50
40
30
20
10
0
100 50 25 12.5 6.3 3.1 1.5 0.8
Conc. of δI6B:ISP in Culture (ug/ml)

FIGURE 4

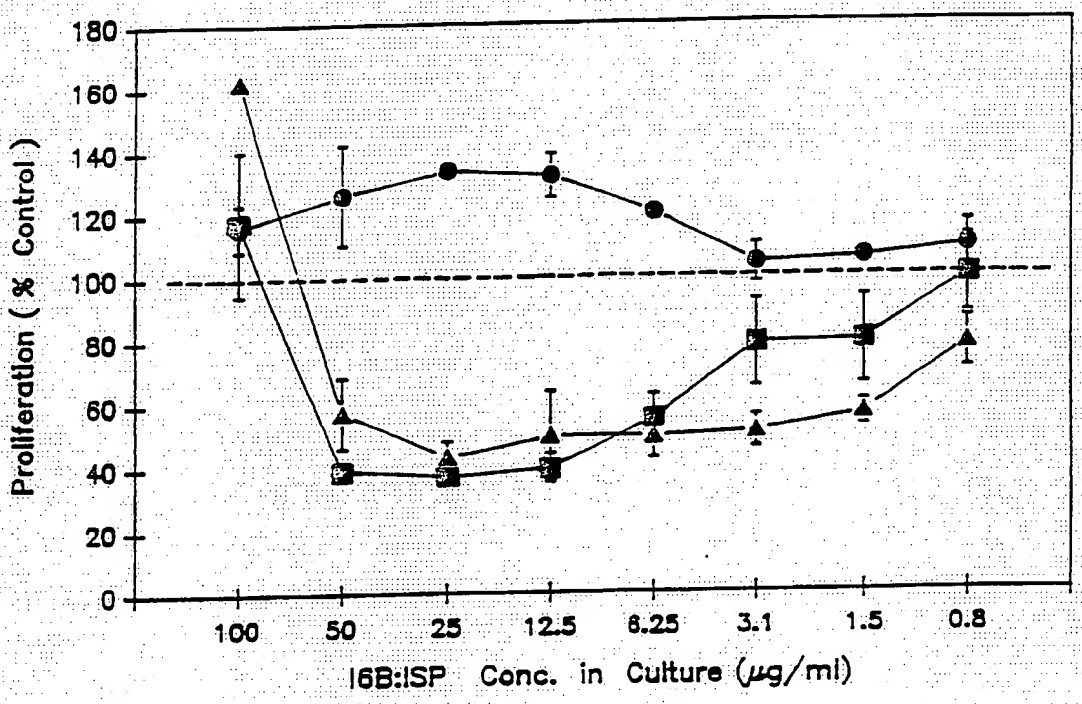
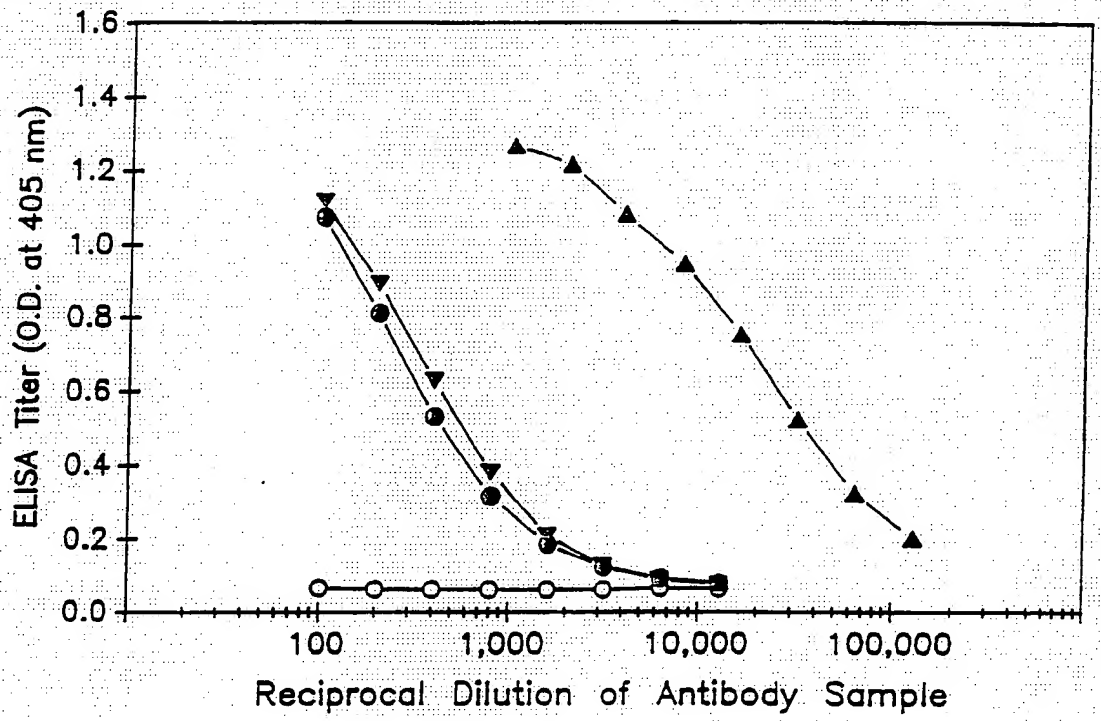

Proliferation (% Control)
180
160
140
120
100
80
60
40
20
0
100
50
25
12.5
6.25
3.1
1.5
0.8
16B:ISP Conc. in Culture (μg/ml)

FIGURE 5


ELISA Titer (O.D. at 405 nm)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
100
1,000
10,000
100,000
Reciprocal Dilution of Antibody Sample

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00176

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4): C07K 7/06, 7/08, 7/10, 7/64, 17/02; A61K 39/12, 42, 385; G01N 33/53; C12N 15/00; C07H 15/12; US: CL: 530/317, 324, 325, 326, 327, 328, 329, 330, 345, 350, 387, 402, 403; 536/27; 435/7, 172.3; 424/85, 88

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/317, 324, 325, 326, 327, 328, 329, 330, 345, 350, 387, 402, 403, 536/27; 435/7, 172.3; 424/85, 88

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

CHEMICAL ABSTRACTS AND BIOLOGICAL ABSTRACTS ONELINE COMPUTER SEARCH

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 4,525,300 (YOSHIDA ET AL) 25 JUNE 1985.	1-44
A	US, A, 4,629,783 (COSAND) 16 DECEMBER 1986	1-44
P,X Y	US, A, 4,663,436 (ELDER ET AL) 05 MAY 1987. SEE CLAIM 1(b) IN PARTICULAR.	3, 39-40, 43-44 4-5
A,P	US, A, 4,689,398 (WU ET AL) 25 AUGUST 1987	1-44
Y	EP, A, 0,114,759 (NICHOLSON) 01 AUGUST 1984. SEE PAGE 8 IN PARTICULAR.	2, 4-5
X Y	NATURE, (LONDON, ENGLAND), VOLUME 312, ISSUED DECEMBER 1984, (PATARCA ET AL), "SIMILARITIES AMONG RETROVIRUS PROTEINS", PAGE 496. SEE FIGURE 2 IN PARTICULAR.	1, 7, 19, 22, 31-44 2, 4-5
X Y	SCIENCE, (WASHINGTON, D.C. USA), VOLUME 230, ISSUED OCTOBER 1985, (CIANCIOLO ET AL), "INHIBITION OF LYMPHOCYTE PROLIFERATION BY A SYNTHETIC PEPTIDE HOMOLOGOUS TO RETROVIRAL ENVELOPE PROTEINS", PAGES 453-455, SEE	1, 7, 19, 22, 31-44 2, 4-5

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search: 24 MARCH 1988

Date of Mailing of this International Search Report: 12 APR 1988

International Searching Authority: ISA/US

Signature of Authorized Officer: CHRISTINA CHAN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
	FIGURE 1 IN PARTICULAR.	
X	J. EXP. MED. VOLUME 159 ISSUED MARCH 1984 (CIANCIOLO ET AL), "HUMAN MALIGNANT AND MITOGEN-TRANSFORMED CELLS CONTAIN RETROVIRAL P15E-RELATED ANTIGEN", PAGES 964-969. SEE PAGE 964 IN PARTICULAR.	25-27
X	CHEMICAL ABSTRACTS, (COLUMBUS, OHIO, USA) VOLUME 105, ISSUED 1986, (MURPHY-CORB ET AL), "ISOLATION OF AN HTLV-III-RELATED RETROVIRUS FROM MACAQUES WITH SIMIAN AIDS AND ITS POSSIBLE ORIGIN IN ASYMPTOMATIC MANGABEYS", SEE PAGE 385, COLUMN 2, THE ABSTRACT NO. 38892_S, NATURE, 1986, 321, 435-7, (ENG).	19-21